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Beyond cadmium accumulation: distribution of other trace elements in soils and cacao beans in Ecuador

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Abstract:

35
36 Since cacao beans accumulate Cd in high levels and restrictions have been imposed on safe
37 levels of chocolate consumption, concern about whether or not cacao trees store other toxic
38 elements seems to be inevitable. Following a previous study in Ecuador examining Cd
39 content in five cacao varieties collected in pristine areas and in places impacted by oil
40 activities, we present here the concentrations of 11 trace elements (TEs) (As, Ba, Co, Cu, Cr,
41 Mo, Mn, Ni, Pb, V and Zn) in soils, cacao tissues (leaves, pod husks, beans) and cocoa liquor
42 (CL). Several TEs showed concentrations in topsoils above the Ecuadorian limits, and may
43 have a mixed natural and anthropogenic origin. Ba and Mo concentrations in cacao tissues are
44 slightly higher than those reported in other surveys, but this was not the case for toxic
45 elements (As and Pb). TE contents are lower in CL, than in beans, except for Pb and Co, but
46 no risk was identified for human health.

47 Compared with control areas, Enrichment Factors were below 2 in impacted areas, except for
48 Ba. Transfer factors (from soils to cacao) indicated that cacao does not accumulate TEs. A
49 positive correlation was found between Cd and Zn in topsoils and cacao tissues for the CCN-
50 51 variety, and between Cd and Ni for the Nacional variety. Identifying patterns of TE
51 distribution and potential interactions in order to explain plant internal mechanisms, which is
52 also dependent on the cacao variety, is a difficult task and needs further research.

53

54 **Keywords:** cacao; trace elements; soil-plant uptake; metal-Cd interactions; Ecuador

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102 **1. Introduction**

103 Cocoa production is one of the main incomes for farmers in tropical countries, and is pursued
104 for high yields and premium quality selection as there are no alternative crops or synthetic
105 products with which to make chocolate (Figuroa-Hernández et al., 2019; Utomo et al., 2016).
106 According to Trade Map (ITC, 2020), in 2019 almost 65% of global cacao exportation was
107 cacao beans (whole or broken, raw or roasted), followed by cocoa butter (fat and oil, 16.9%),
108 cocoa powder not containing sugar (16.9%) and cocoa shells, husks, skins and other waste
109 (1.8%).

110 Cacao is cultivated on tropical lands in West Africa, Central and South America, the West
111 Indies and Asia (Kongor et al., 2016). Cacao from Ecuador is famous for its fine flavor which
112 is found in several varieties, the two most important being “Nacional Fine Flavor”, which is in
113 great demand in Europe for producing the finest chocolates, and the “CCN-51 variety”, which
114 is very productive and more resistant to tropical diseases (Beg et al., 2017; Vargas Jentsch et
115 al., 2016). Although its pod morphology and tree architecture are distinct from the traditional
116 Nacional genotypes, CCN-51 is sometimes mistaken for being of Nacional lineage (Boza et
117 al., 2014).

118 In Ecuador, just under 20 ha of cacao plantations (nearly 76% of the total cultivated) are
119 located on small farms, where it is specifically family labor. Up to 90% is managed under
120 traditional systems, especially for fine flavor cocoa, which means a lesser use of chemical
121 fertilizers, machinery and irrigation units compared to CCN-51 farming (Saravia-Matus et al.,
122 2020).

123 It is now largely admitted that cacao can contain trace elements (TEs) that mainly accumulate
124 in the beans shell (Yanus et al., 2014). Some are required by plants as micronutrients (Mn,
125 Cu, Zn, Ni and Mo) but others are considered toxic for human health, especially As, Cd, Cr
126 and Pb (Kabata-Pendias, 2011; Page and Feller, 2015; Assa et al., 2018). These elements are
127 obtained directly from the soil and/or during the production process (Assa et al., 2018).

128 The distribution of elements in cacao beans has recently been studied using LA-ICP-MS bio-
129 imaging techniques, which showed that both essential elements (Mg, P, K, Cu and Zn) and the
130 toxic ones (Pb and Cd) were enriched in the seed shell of the processed cacao bean (Thyssen
131 et al., 2018).

132 Special attention is being paid to Cd, as the European Union has enforced stricter upper limits
133 for its content in cocoa-based products since January 1st, 2019 (European Commission, 2014).
134 In Latin America high levels of Cd have been found in cacao beans from Ecuador (Argüello

135 et al., 2018; Barraza et al., 2017; Chavez et al., 2015), Honduras (Gramlich et al., 2018) and
136 Peru (Arévalo-Gardini et al., 2017; Zug et al., 2019). Differences in Cd concentrations in the
137 edible parts may be explained by cultivar or genotype differences in its uptake, root-to-shoot
138 translocation and above-ground partitioning (Barraza et al., 2019; Engbersen et al., 2019;
139 Lewis et al., 2018; Mengist et al., 2017; Moore et al., 2020). Cd is highly toxic for human
140 health, but it is certainly not the only element which accumulates in cacao beans, and more
141 investigation is needed regarding other TEs to better understand the potential risks involved
142 for human health and the possible combined effects of the co-accumulation of toxic metals.
143 Arévalo-Gardini et al. (2017) reported that concentrations of Cr, Ni and Pb in cacao beans and
144 leaves in Peru varied between sampling sites and genotypes. High levels of Ni have also been
145 found in raw cacao and in chocolate (7.6 and 4.5 mg kg⁻¹ respectively) made with beans from
146 Ecuador and the Dominican Republic (Kruszewski et al., 2018). In a recent review of
147 exposure to toxic metals, Anyimah-Ackah et al. (2019) reported variable and sometimes high
148 concentrations -compared to international regulations- of As, Hg and Pb in cacao beans and/or
149 cocoa-based food. A health risk assessment conducted in Saudi Arabia concluded that the
150 estimated daily intake of heavy metals (Al, As, Co, Cu, Cd, Cr, Hg, Mn, Ni and Pb) from the
151 consumption of chocolate and cocoa products was below the US EPA safe standards,
152 however, the hazard index for a specific group of cocoa products was almost twice as high as
153 the recommendations, suggesting that frequent consumption may result in adverse non-
154 carcinogenic health effects in consumers (Salama, 2019).

155 Interactions between Cd and several micronutrients have been observed, notably with Cu, Fe,
156 Mn, Ni, Se and Zn, producing depressing or enhancing effects in plant nutrition and
157 physiology (Kabata-Pendias and Szeke, 2015; Khaliq et al., 2019), and then potential
158 cumulative effects on human health after consumption. The transport of essential TE across
159 membranes at long-distance, and intracellular sequestration, is often carried out by high
160 specific proteins, but when ions have similar chemical properties they can share the same
161 routes. This also applies to non-essential TEs (Andresen et al., 2018; Van der Ent et al.,
162 2013).

163 There thus seem to be several questions about the amounts of TEs -potentially toxic or not- in
164 cacao beans, motivating researchers to conduct more studies that can generate reliable data
165 about concentrations, sources, transfer and accumulation within the soil-cacao system.

166 The aims of our study are thus to: (i) determine the distribution of TEs in the soils of more
167 than 30 Ecuadorian cacao small-scale farms, (ii) understand the distribution of TEs in cacao
168 plant tissues (leaves, pod husks and beans) and in cocoa liquor, in specific varieties of cacao,

169 (iii) determine if these elements are transferred from soil and later bioaccumulated, in order to
170 better assess the possible health implications, and finally (iv) identify the potential
171 interactions between Cd and other TEs.

172

173 **2. Materials and Methods**

174

175 **2.1 Study Area**

176 Cacao samples and their associated topsoils were collected between 2014 and 2016 as
177 described in Barraza et al. (2017) from a total of 31 small-scale farms (Fig. SI-1, ESI) located
178 in the Ecuadorian provinces of Orellana and Sucumbíos in the northern Amazon region
179 (NAR); in Morona-Santiago, in the southern Amazon (MS); and along the north and south
180 Pacific coast, in Esmeraldas (NPC) and Manabí (MC), respectively.

181 The choice of these sites was based on the presence or absence of oil facilities (to serve as
182 control areas), as a part of the MONOIL research program (Environmental Monitoring,
183 Health, Society and Oil in Ecuador).

184 Five cacao varieties were collected: Nacional, CCN-51, Forastero, Zamora and Nativo
185 amazonico (native) (Fig.SI-1, ESI). Two cocoa liquor (also known as cocoa mass) samples
186 were also collected in Pacayacu (Sucumbíos province, NAR) in order to determine the
187 elemental concentrations in the final product. Detailed information about cocoa liquor
188 elaboration is provided in Barraza et al. (2017).

189

190 **2.2 Soil and cacao sampling and preparation**

191 One mature cacao pod and ten green leaves were selected for each sampled tree (2 or 3 trees
192 for each point) and mixed together. The associated soil samples were collected as composites
193 around each tree, from 0 to 60 cm, and in some sites to 100 cm depth, which corresponds to
194 the natural geochemical background. Note that the present study focused on the topsoil (0-20
195 cm) as results have been already presented and discussed for the deepest layers in Barraza et
196 al. (2018). It has been observed that most cacao roots are situated at a depth of 0-20 cm
197 (Kummerow et al., 1982; Niether et al., 2019).

198 Cacao beans were separated from their pod husks, and air-dried for three days before being
199 oven-dried at 40 °C for two days, as were the leaves. All cacao tissues were ground with
200 liquid nitrogen using an agate mortar and pestle. Soil samples were air-dried, sieved at 2 mm,
201 and ground with an agate bench ring mill.

202

203 **2.3 Soil physico-chemical properties and soil identification**

204 Pedological and physico-chemical properties such as pH, cationic exchange capacity (CEC)
205 and total organic carbon (TOC) were reported for each sampling site in Barraza et al. (2017).
206 Briefly, soils were divided into three categories: inceptisols (NAR, MS), alfisols (MC) and
207 entisols (NPC) according to the USDA classification (USDA, 1999). pH was acidic (between
208 4 and 6.8) in the NAR and MC regions, and slightly neutral to basic in the NPC and MC areas
209 (between 7.1 and 8). Low CEC (ranging from 3 to 18 cmol kg⁻¹), typical of tropical soils, was
210 related to soil enrichment in secondary minerals such as gibbsite, goethite and kaolinite (Juo
211 and Franzluebbbers, 2003). Contents in TOC were variable, with low values (below 1 g kg⁻¹) in
212 the deepest soil layers (up to 80 cm), and higher values (above 40 g kg⁻¹) in upper layers (0-60
213 cm).

214

215 **2.4 Determination of trace elements concentration**

216 Eleven trace elements were identified in the collected samples: As, Ba, Co, Cu, Cr, Mo, Mn,
217 Ni, Pb, Zn and V. These elements were chosen because of concerns regarding their
218 environmental effects, and the potential impact of oil activities in Ecuador (led by the French
219 MONOIL research program).

220 Dried soil and cocoa liquor (CL) samples (0.1 and 0.125 g respectively) were mineralized -in
221 a clean lab- in a high pressure microwave (Mars 5 CEM[®]) with a solution of 11 ml of aqua
222 regia (9:2) and 3 ml of HF (for soils) or 9 ml HNO₃, 1 ml H₂O₂ and 0.2 ml HF (for cocoa
223 liquor). Reagents were of Suprapur[®] quality or sub-boiled (HNO₃ and HCl). Solutions were
224 then completely evaporated at 60 °C on a hotplate and the dried residue was resuspended by
225 adding 0.5 ml HNO₃.

226 Dried plant samples (0.125 g of leaves, pod husks or beans) were digested on a hotplate
227 system (Digiprep, SCP Science[®]) using 5 ml of HNO₃ and 5 ml of H₂O₂. Digestion solutions
228 were diluted with high purity deionized water (Milli-Q Element[®]) and filtered through a clean
229 0.22 µm cellulose acetate membrane.

230 Mineralized solutions were finally diluted with HNO₃ 0.37N prior to taking concentration
231 measurements by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent[®] 7500
232 CE, GET laboratory).

233 Each batch of samples included a lab blank, certified reference material and some replicates.
234 The limits of detection (LD) and quantification (LQ) were calculated as three and ten times
235 the standard deviation (SD) of lab blanks. Three certified reference materials (from the
236 National Institute of Standards and Technology): SRM 2709 "San Joaquin soil", SRM 1515

237 “Apple leaves” and SRM 2384 “Baking chocolate”, were used to validate the analytical
 238 methods. Recoveries between 75 to 100% were obtained for these elements (Table SI-1, ESI).

239

240 **2.5 Global Enrichment and Transfer Factors**

241 The global enrichment factor (GEF), as defined in Schreck et al. (2014), was calculated to
 242 determine the degree of metal accumulation in cacao beans grown in areas impacted by oil
 243 activities with respect to a control area, according to the following equation :

244 *Equation (1):*

$$245 \quad \text{GEF} = \frac{[\text{TE}] \text{ cacao beans grown on contaminated sites}}{[\text{TE}] \text{ cacao beans from control site}}$$

246
 247 where [TE] is the concentration of each trace element expressed in mg kg⁻¹.

248 Trace element concentrations in cacao beans from the control site were calculated as the mean
 249 value of Samples 3 and 4, corresponding to the CCN-51 variety, located in areas not affected
 250 by extractive activities, in Morona-Santiago (MS) as described by Barraza et al. (2017). This
 251 MS area is free from mining or oil production, agriculture is not intensive and crops are not
 252 irrigated. Even if the CCN-51 variety differs from the Nacional, the latter is often used as a
 253 parent in crosses in the breeding programs in Ecuador (Boza et al., 2014). As previously
 254 noted, it is also common to find Nacional and CCN-51 natural hybridization on local farms.

255 Trace elements uptake and accumulation from soil to plant, known as bioaccumulation or the
 256 transfer factor (TF), and described by Nirola et al. (2015) and Schreck et al. (2012), were also
 257 calculated (Equation 2) in order to determine a possible enrichment in TE in cacao tissues
 258 (leaves, pod husks and beans) relatively to the topsoil (0-20 cm).

259 *Equation (2):*

$$260 \quad \text{TF} = \frac{[\text{TE}] \text{ cacao tissues}}{[\text{TE}] \text{ soil}(0-20 \text{ cm})}$$

261
 262

263 TF>1 indicates that cacao tissues are enriched in an element, TF around 1 indicates the
 264 relatively indifferent behavior of the plant towards this element, and TF<1 shows that the
 265 plant excluded this element from its mineral nutrition and thus from its uptake (Mingorance et
 266 al., 2007).

267

268 **2.6 Statistical analysis**

269 The R® software version 3.1.1 was used for graphics and statistical analyses. As our data did
 270 not follow a normal distribution, Spearman’s correlation (r_s) was used to measure the strength

271 and relationship between soil properties, TE concentrations in the topsoil and in cacao leaves,
272 pod husks and beans. In addition, Cd concentrations for the same type of samples were
273 included and taken from Barraza et al. (2017). For this purpose, only CCN-51 (13 samples)
274 and Nacional varieties (17 samples) were considered, independent of the sampling site.
275 Two ranges were fixed to evaluate correlations: a moderate correlation for the r_s range
276 between 0.5 and 0.64, and a strong one for r_s up to 0.65.

277

278 **3. Results and Discussion**

279

280 **3.1 Distribution of Trace Elements in Soils**

281 In all sampling sites, topsoils included at least two elements above the Ecuadorian threshold
282 for TEs (Table SI-2, ESI). In the oil extraction area (NAR), most showed concentrations
283 above the national recommendations for Ba, V, Zn, Cr, Cu and Ni (Co and Mo in a lesser
284 extent). Only V was above the limits according to international guidelines for soil quality in
285 agricultural areas in Canada (Canadian Council of Ministers of the Environment, 2018) and
286 the European Union (The European Commission, 1986) (Table SI-2, ESI).

287 National regulations have different limits depending on land use (residential, commercial,
288 industrial and agricultural) but none take into account TE mobility in special environments
289 such as plant systems and water corps (Antoniadis et al., 2019). There is no international
290 agreement with respect to the TEs that have to be regulated, as their natural content varies
291 according to soil type, weathering level and, physiographic regions (Da Silva Costa et al.,
292 2017).

293 The farms with the highest concentrations were located in the NAR, close to oil facilities (e.g.
294 platforms, oil pits). Concentrations of Ba, V and Ni ranged from 31-785, 86-284 and 8.1 to 37
295 mg kg⁻¹ respectively, whereas their concentrations in the NPC (in the refinery zone) were less
296 variable, with mean values of 337 ± 66 and 144 ± 10 mg kg⁻¹ for Ba and V respectively. Ni
297 showed concentrations between 35 to 157 mg kg⁻¹ in the NPC (Fig. 1, Table SI-2).

298 Our previous study in the NAR (Barraza et al., 2018) has already discussed the potential
299 sources of trace elements (a combination of parent rock material, volcanic emissions, oil
300 activities, agrochemical products, among others) and explained their high spatial variability,
301 which also seems to be true in the MS and NPC areas. High concentrations of Ba, V and Ni
302 were measured in crude oil samples (133 ± 5 , 305 ± 36 , and 99 ± 1 mg kg⁻¹, respectively),
303 however, it may not only be oil activities behind high levels of some elements recorded in

304 these areas, especially considering we studied the farms located in MS and MC, free of oil
305 extraction or refining.

306 It's now well known that a combination of natural and anthropogenic sources can release
307 significant amounts of TEs into the environment. As an example, intensive agriculture -as
308 developed in MC and in some farms in the NAR- using P-fertilizers, herbicides, pesticides
309 and livestock manure, can increase contents of As, Cd, Cu, Cr, Zn in soils (Kabata-Pendias,
310 2011; Shi et al., 2018). In addition to the intense weathering and leaching observed in the
311 tropics, combined with clay content, we might expect low pH values, low OM and high levels
312 of Al-Fe or even Mn oxides to result in the relatively high mobility and bioavailability of TEs
313 in soils, such as As, Ba, Cd, Co, Cu and Pb (Cappuyns, 2018; Rieuwertts, 2007; Souza et al.,
314 2018).

315 Arévalo-Gardini et al. (2016) reported a wide range of concentrations for Mn (82 to 1275 mg
316 kg^{-1}), Zn (3.76 to 112 mg kg^{-1}), Cu (2.43 to 54 mg kg^{-1}), Ni (1.64 to 43 mg kg^{-1}) and Pb (5.62
317 to 22 mg kg^{-1}) in topsoils in cacao plantations in eight provinces in Peru, depending on the
318 cocoa plantation's location. Elements such as Cu and Ni were more concentrated in the
319 deepest soil layers (until 80 cm) unlike Mn, Zn and Pb. It has been already reported that these
320 elements, especially Mn and Pb, are more concentrated in topsoils, rather than leaching down
321 from the upper layers (Da Silva Costa et al., 2017; Kabata-Pendias, 2011) due to their low
322 mobility in the soil column. The concentrations reported by Arévalo-Gardini et al. (2016) in
323 Peru were in the same range as those found in our study, however the levels recorded for Ni
324 and Cu (8-157 and 12-92 mg kg^{-1} respectively; Fig. 1, Table SI-1) are slightly higher in our
325 study in Ecuador.

326

327 **3.2 Trace elements concentrations in cacao tissues and cocoa liquor, and potential** 328 **health implications**

329 The concentration of TEs in cacao tissues (leaves, pod husks and beans) followed the same
330 tendency observed for Cd in our previous study (Barraza et al., 2017): they were highest in
331 leaves, then in pod husks and finally in beans (Fig. 2; Table SI-3). Concentrations of elements
332 such as As, Co, Cr, V and Pb, were below the detection limits in cacao tissues.

333 Only Co and Pb were detected in cocoa liquor samples (Table 1).

334

335 **3.2.1 In cacao leaves**

336 Manganese concentrations ranged from 12 (NPC, Nacional variety) to almost 1960 mg kg^{-1}
337 (NAR, Nacional variety). The mean concentration of Ba (from all sites and all varieties) was

338 $115 \pm 19 \text{ mg kg}^{-1}$, with the highest value reaching 354 mg kg^{-1} in the NAR for the Nacional
339 variety (Table SI-3).

340 Crystals of BaSO_4 were observed by environmental scanning electron microscopy with
341 energy dispersive x-ray spectroscopy (ESEM-EDX; results not shown) in washed and
342 unwashed cacao leaves from some sampling sites located near open flares in oil platforms in
343 the NAR. These results could suggest a link between oil activities and Ba concentrations in
344 cacao tissues, either through atmospheric deposition and/or root uptake, as BaSO_4 is used as a
345 weighting agent during drilling (Oskarsson, 2015).

346 Concentrations of Zn varied between 17 and 263 mg kg^{-1} , with the highest value also recorded
347 for the Nacional variety. Copper and Ni concentrations in cacao leaves were between 0.65 and
348 nearly 40 mg kg^{-1} . The highest concentrations for both elements were found in the NAR for
349 the CCN-51 variety. Molybdenum concentrations ranged from 0.05 (in MS) to 8.44 mg kg^{-1}
350 (in MC) (Table SI-3).

351 Concentrations of Zn and Mn were 1.4 to 4.5 times higher in our study than those reported by
352 Arévalo-Gardini et al. (2017), which mainly included CCN-51 genotypes collected in Peru.
353 However, also in Peru, Huamani et al. (2012) recorded Mn and Zn concentrations
354 approximately 2.7 to 5.4 times lower than values found for cacao leaves in Ecuador. Arévalo-
355 Gardini et al. (2017), however, found Cu and Ni mean concentrations similar to those found
356 in our study.

357

358 **3.2.2 In pod husks**

359 Mean manganese concentrations varied from 11 ± 2 to $136 \pm 26 \text{ mg kg}^{-1}$ in NPC and MS,
360 respectively (Fig. 2). Concentrations of Zn reported by Gramlich et al. (2017 and 2018) in
361 Latin America were of 42.9 ± 1.6 and $45.8 \pm 1.4 \text{ mg kg}^{-1}$, respectively in Bolivia and
362 Honduras, and similar to the values found in our study, in the NPC ($41.5 \pm 5.9 \text{ mg kg}^{-1}$) and
363 MC areas ($47 \pm 4.7 \text{ mg kg}^{-1}$).

364 Barium concentrations varied from 2 (MC) to 98 mg kg^{-1} (NAR) in the CCN-51 cacao variety.
365 The highest concentration of Cu (24.20 mg kg^{-1}) was found in the NAR, also in the CCN-51
366 variety (Table SI-3). Mean nickel concentrations ranged from 3.10 to 6.27 mg kg^{-1} . The
367 highest mean Mo concentrations were 0.58 and 0.24 mg kg^{-1} for Nacional and CCN-51,
368 respectively (Table SI-3).

369

370 **3.2.3 In cacao beans**

371 Mean Zn concentrations in cacao beans varied from 51 to 65 mg kg⁻¹ in the NPC and in the
372 MS, respectively (Fig. 2). The highest concentration of Mn (176 mg kg⁻¹) was found in the
373 NAR for the Nacional variety (Table SI-3) in a specific fruit affected by a disease caused by a
374 fungus (*Moniliophthora roreri*), one of the most serious problems for cacao production in
375 Latin America (Bailey et al., 2018).

376 Barium concentrations ranged between 1.2 and 45.6 mg kg⁻¹ for the Nacional variety, in MC
377 and in NAR, respectively (Table SI-3). In the NAR, mean concentration of this element was
378 16 ± 3 mg kg⁻¹ (Fig. 2). Mean Nickel and Cu concentrations for each variety ranged from 1 to
379 14 and 5 to 37 mg kg⁻¹ respectively, with the highest values in the Nacional variety (Table SI-
380 3).

381 In Esmeraldas (NPC) and Manabi (MC), Romero-Estévez et al. (2019) found that the highest
382 concentrations of Ni were 8.53 and 6.03 mg kg⁻¹ respectively, also for Nacional, CCN-51 and
383 blended cacao (i.e. Criollo, Forastero and Trinitario) samples. Compared to our results for the
384 same locations and varieties, Ni mean concentrations were 9.81 ± 1.52 and 8.11 ± 1.02 mg kg⁻¹
385 (calculated from data summarized in Table SI-3). The same authors found non-negligible Pb
386 contents in beans collected in nine provinces of Ecuador (mean 1.43 mg kg⁻¹), while they
387 were all under the LQ in our study.

388 In a recent study of the elemental composition of cacao beans (Forastero variety), Bertoldi et
389 al. (2016) reported concentrations of Cu and Ni in samples collected in South America close
390 to our values. Ba (22.2 ± 12.6 mg kg⁻¹), Mn (35.4 ± 11.6 mg kg⁻¹) and Zn (73.4 ± 60.8 mg kg⁻¹)
391 concentrations in cacao beans for West Africa and Asia were also similar to our values for
392 NAR and MS sampling areas. Assa et al. (2018) demonstrated Cu concentrations in cacao
393 beans (without shell) of 10 to 19 mg kg⁻¹ for three cacao clones in Indonesia, which is 1.5
394 times lower than the content found in our study. Kruszewski et al. (2018) showed
395 concentrations of Ni in cacao beans with shell from Ecuador and the Dominican Republic of
396 2.32 ± 0.45 and 7.62 ± 0.51 mg kg⁻¹, respectively. The Nacional variety had, considering all
397 sampling sites, a similar mean concentration (6.91 ± 3.62 mg kg⁻¹) to those from Dominican
398 Republic, but almost half that of the CCN-51 variety.

399 In the same way, mean Mo concentration in our study reached 0.37 ± 0.19 mg kg⁻¹ for CCN-
400 51 variety, similar to the values recorded by Bertoldi et al. (2016) in beans from South
401 America for the Forastero variety. Conversely, Yanus et al. (2014) reported very low Mo
402 concentrations of 0.032 ± 0.005 mg kg⁻¹ in beans from an unknown origin imported by Israel.

403

404 Based on these results, we cannot confirm that TEs concentration in cacao tissues are strongly
405 related to a variety effect with any certainty, even if some differences have been observed
406 with respect to Cd concentrations, which seem to be higher in the Nacional variety than in
407 CCN-51 (Argüello et al., 2019; Barraza et al., 2017).

408

409 **3.2.4 In cocoa liquor**

410 Cocoa liquor is a pure cocoa mass in liquid form produced from the cocoa nibs (fermented,
411 cleaned and unshelled roasted cacao beans) through heat generation during the grinding
412 process. It contains both cocoa solids and cocoa butter in roughly equal proportions to the end
413 product (Beg et al., 2017). For didactic purposes, our results are mainly compared to dark
414 chocolate, as cocoa liquor is closer to the final product than the beans presented in the
415 previous section.

416 Elemental analysis performed in 10 dark chocolate samples (70-75% of cocoa solids)
417 purchased at local markets in Serbia (Mrmošanin et al., 2018) showed mean contents of 23.3,
418 14.2, 13.4, 5.37 and 3.21 mg kg⁻¹ for Zn, Cu, Mn, Ba and Ni, respectively. Of these elements,
419 only Mn and Ba concentrations were similar in our study, and Zn, Cu and Ni were twice as
420 high in our CL samples. Kruszewski et al. (2018) reported Ni concentrations of 5.72 ± 0.13
421 and 5.87 ± 0.10 mg kg⁻¹ in cocoa liquor samples from multiple producers (chocolate factories
422 in Poland). These values were in accordance with our sample CL-1, but 1.5 lower than our
423 sample CL-2 (Table 1).

424 Eight dark chocolate samples (> 60% of cacao solids) elaborated with cacao beans from South
425 America (Bertoldi et al., 2016) presented Mn and Ni concentrations similar to those in our
426 study, whereas Zn and Cu were half as much (25.9 ± 10.2 and 13.3 ± 4.7 mg kg⁻¹
427 respectively). By contrast, Ba mean concentrations were almost the double those in our
428 samples (7.93 ± 1.78 against 4.9 ± 1.84 mg kg⁻¹). Recently, Vanderschueren et al. (2019)
429 found mean concentrations of 29 ± 8, 13.6 ± 5.9, 13 ± 4.2, 4.9 ± 3.3, 3.2 ± 1.8 and 0.24 ± 0.1
430 mg kg⁻¹ for Zn, Cu, Mn, Ba, Ni and Mo, respectively, in chocolate bars from South America
431 (including 34 samples from Ecuador) with a median of 70% cacao solids, which were 2 to 3
432 times lower than our results, except for Mn (Table 1).

433 If we calculate mean TE concentrations in raw beans – mixing Nacional and CCN-51
434 varieties samples in Sucumbíos (farms 13 to 31) which is the same region as where the CL
435 samples were collected – Cu, Mo and Ni showed similar values while Mn, Zn and Ba
436 concentrations were higher in CL compared with raw beans. This is in agreement with Yanus
437 et al. (2014) who found increased Mn, Co and Mo in cocoa powder with respect to raw beans

438 and less in the cocoa butter. These differences might be explained by the fact that some
439 elements are more concentrated in the shells, which is removed to elaborate cocoa products,
440 than in the bean core itself (Thyssen et al., 2018; Yanus et al., 2014).

441 Conversely, for Co and Pb -which were not detected and/or were below the LQ in raw beans-
442 the concentrations found in CL samples would suggest either an enrichment or a slight
443 contamination during manufacturing or lab analysis. The Pb concentrations measured in our
444 CL samples (between 6 and 10 $\mu\text{g kg}^{-1}$; Table 1) were a tenth of the mean observed in several
445 chocolate brands consumed, for example, in Israel (Yanus et al., 2014) and 100-fold lower
446 than the Pb concentrations measured in Ecuadorian cocoa beans (Romero-Estévez et al.,
447 2019).

448 Finally, in terms of toxic element content and associated health risks, there are no current or
449 updated regulations for TEs other than Cd in chocolate, but several limits are proposed in the
450 CODEX Alimentarius. A report of the Codex Committee (FAO and WHO, 1997) indicates
451 that the presence of Pb and Cu should not exceed 1 or 2 and 20 or 35 mg kg^{-1} respectively in
452 food, depending on the content of sweeteners (with vs without) and the percentage of cocoa
453 solids in chocolate. Assuming this scenario, both CL samples had levels above the
454 recommendations for Cu (in chocolate products, with the exception of the unsweetened ones).
455 Co, Mn, Mo and Zn are essential for humans, and knowing that their toxicity (very low in
456 some cases) is related to other relevant exposure pathways (inhalation, water ingestion or
457 dermal contact), none represent a real threat to the health of regular chocolate consumers.
458 Some of the estimated safe or adequate daily dietary intakes (Co: 0.005-0.029 mg; Mn: 2-5
459 mg; Mo: 0.075-0.250 mg; Zn: 8-11 mg, as reported by Kabata-Pendias and Szteke, 2015) are
460 not exceeded even when consuming a bar of dark chocolate (~110 g) per week.

461 The results are the same for non-essential elements such as Ba, with an estimated daily dietary
462 intake of 0.75 to 1.33 mg and a tolerable daily intake of 0.2 mg kg^{-1} BW, and Ni with a
463 tolerable daily intake of 12 $\mu\text{g kg}^{-1}$ of BW (Kabata-Pendias and Szteke, 2015), they do not
464 pose any threat to human health.

465

466 **3.3 Influence of biotic and abiotic factors on TE uptake in cacao**

467 The biochemical cycles of TEs in plants are governed by several external and internal factors
468 including soil properties (mobility and fate), the behavior of elements in soil solution
469 (competition, interactions), plant physiology (transport, genotypes), and the rhizosphere
470 microbiota (Antoniadis et al., 2017; Kabata-Pendias, 2011). Each factor will be discussed in
471 more detail below.

472

473 **3.3.1 Plant variety**

474 The uptake capacity of TEs varies between genotypes of the same plant species as observed in
475 Chinese cabbage (Mi et al., 2019), asparagus (Conversa et al., 2019) and rice (Fahad et al.,
476 2019). Significant differences between genotypes have been found in cacao with regard to
477 physiological traits such as leaf chlorophyll content (Daymond and Hadley, 2004), stomatal
478 conductance (Daymond et al., 2011), biomass and plant growth (Lahive et al., 2019).

479 As already mentioned in Section 3.2, similar concentrations of TEs have been found in the
480 CCN-51 and Nacional varieties (which represents 88% of the samples), suggesting that this
481 factor might not have any strong effect on TE accumulation in cacao tissues. Correlations
482 between elements and soil parameters could suggest some differences, however, which will
483 be discussed in Section 3.4.

484

485 **3.3.2 Influence of anthropogenic activities**

486 Based on GEF calculations (Fig. 3) that allow comparisons with pristine sites in terms of
487 extractive activities, the cacao beans in our study appear to be mainly enriched in Ba (66% of
488 the samples), Zn (62%), Ni (59%), Mn (28%) and to a minor degree, Cu (3%). None of the
489 samples displayed a GEF up to 1 for Mo.

490 The highest GEFs (8.2 and 11.8) were found for Ba in cacao beans (Nacional and CCN-51
491 varieties, respectively) collected in two farms located in the NAR, in areas well-known for the
492 large record of oil spills, oil waste disposal and gas open flares emissions (Sampling Sites 6
493 and 9 respectively).

494 The GEF for Cd in the same samples (Barraza et al., 2017) was 4 to 18 times higher than the
495 values for the TEs presented here, explaining among other things, why Cd is of high concern
496 nowadays.

497

498 **3.3.3 Effect of soil and elements properties**

499 The transfer factors for the CCN-51 and Nacional varieties were higher in leaves, and then in
500 pods or beans, except for Cu (beans>pod husks ~ leaves). TFs from soil to leaves for essential
501 elements such as Zn, Cu, Mo and Ni were also lower than those for Mn and Ba, whereas TFs
502 in beans were higher for Mo, Cu and Zn (Table 2).

503 As the TF depends on both soil properties and plant characteristics, it is closely related to the
504 concept of the bioavailability of TEs, because for a given element, it records only the portion
505 that is able to be uptaken by the plant (Antoniadis et al., 2017).

506 The TF values also showed that after translocation to the shoots, the redistribution from leaves
507 to pods and then to beans seems to be limited. As discussed by Page and Feller (2015), some
508 elements within shoots can be redistributed from senescing leaves via the phloem to sinks
509 such as growing vegetative parts and maturing fruits. As recently observed for three different
510 cacao cultivars in Honduras, however, a substantial amount of Cd would be directly loaded
511 from stems and branches into the beans, without prior passage through leaves, which implies
512 a substantial xylem-to phloem transfer in the woody parts of cacao trees (Engbersen et al.,
513 2019). Our results therefore also suggest different mechanisms for the transfer of other TE
514 and their distribution within cacao tissues.

515 Usually Mn, Cu, Mo and Ni are quite mobile in soil and readily taken up by plants, whereas
516 Cd and Zn are very mobile and easily bioaccumulated (Kabata-Pendias, 2011). Cadmium and
517 Zn share very similar chemical properties and it has been shown that a relative deficiency in
518 Zn in the soil may lead to increased Cd uptake as they compete for the same transport
519 membranes (Sarwar et al., 2010).

520 Our results only found a Zn enrichment in a few samples ($TF_{\text{leaves}} > 2$, both varieties), as Zn
521 could be widely leached in depth in these kinds of tropical soils with low pH values and high
522 weathering processes. Several factors (P compounds, clay fractions, solved organic matter)
523 can also hold Zn quite strongly, reducing its phytoavailability (Kabata-Pendias and Szteke,
524 2015).

525 The mobility of Cu decreased at pH 7-8, and its phytoavailability was affected by Cu species
526 in soil solutions rather than by total concentration (Kabata-Pendias and Szteke, 2015). The
527 Cu-TF also remained below 2 (Table 2), indicating that cacao trees only seem to uptake the
528 Cu quantities needed for physiological functions.

529 Nickel is highly phloem-mobile and can be repeatedly redistributed throughout vegetative
530 growth and the reproductive phase (Page and Feller, 2015), but, in some samples of cacao, it
531 seemed to remain concentrated in leaves and pod husks ($TF = 1.40$ in these two tissues, for
532 both varieties), without reaching higher levels, as in Ni-accumulators (Rascio and Navari-
533 Izzo, 2011). However, contrary to the assumption that Ni availability increases at pH less
534 than 6.5 (Shahzad et al., 2018), our results did not show a clear trend in the increase of Ni
535 concentration in cacao tissues, nor its TFs.

536 The behavior of Mo in soils has been extensively studied because it holds a unique position
537 among other micronutrients. In soils with $pH < 5.5$, Mo is hardly available to plants, whereas
538 it is readily mobilized in alkaline soils (Kabata-Pendias and Szteke, 2015). These statements

539 are in agreement with our results: the lowest Mo-TFs were observed in soils with a pH < 5.9,
540 although some high TFs were found in leaves (see Sampling Site 23).

541 A TF_{leaves} of 14.29 and 5.04 were found for the Nacional variety in the sampling site 11
542 (NAR) for Mn and Ba respectively, where the soil pH was 4.4. Both elements are readily
543 phytoavailable in acid soils (Abbasi et al., 2016; Kabata-Pendias, 2011). In tropical soils Ba
544 generally occurs in the mobile form of hollandite - associated with Mn (hydro)oxides- and
545 easily available to plants (Cappuyns, 2018; Kabata-Pendias, 2011)

546 An enrichment of Mn was observed in leaves for one sample affected by the frosty pod
547 disease (Sampling Site 7, Nacional variety). This essential element is known to have positive
548 effects in enhancing plant tolerance against fungal diseases (Eskandari et al., 2018) and on the
549 mitigation of Cd toxicity in the CCN-51 cacao genotype (Oliveira et al., 2020). A continued
550 accumulation of Mn in the oldest leaves, with a limited translocation to young organs, results
551 from the translocation of Mn from the roots in some plants (Kabata-Pendias, 2011; Xu et al.,
552 2006).

553 Close attention should be paid to the transfer factors calculated here for leaves and pod husks,
554 since concentrations of TEs could also result from dust depositions and not necessarily only
555 from root uptake and redistribution within the plant. The foliar transfer of metals is either not
556 present or in some cases appears as the main pathway for uptake in severe contaminated sites
557 (Shahid et al., 2017). This could be the case for certain cacao farms located close to oil flares,
558 since TE concentrations in air seem to be high for elements such as Ba (Barraza et al., 2020).

559

560 **3.4 Insights for patterns of distribution and potential interactions between TEs in the** 561 **soil-cacao system**

562 Synergetic and antagonist effects are common behaviors among TEs in plants where different
563 chemical species of the same element can affect each other, or when one or several elements
564 alter the distribution of the others in plant tissues. The best ways to try to identify these effects
565 in cacao, together with its mineral nutrition status, is through controlled conditions in
566 greenhouse experiments (Blommaert, 2019; Pereira de Araújo et al., 2017) or directly in the
567 field collecting samples from a well-defined study area (Marrocos et al., 2020; Quintino R. de
568 et al., 2020).

569 In this study, we were confronted with the reality of the field sampling, where crop conditions
570 were not controlled: (i) cacao varieties were classified based on the phenotype information
571 supplied by the farmers, and (ii) TEs concentrations in Ecuadorian tropical soils displayed
572 high spatial variations due to natural or anthropogenic perturbations (Barraza et al., 2018).

573 Nevertheless, the combination of TF calculations and correlation matrices, demonstrated
574 some differences in the pathways for the uptake of TEs.

575

576 **3.4.1 Correlations between Ba, Cu, Mn, Mo, Ni and Zn**

577 In the Nacional variety, Ni concentrations in leaves, pods and beans were strongly correlated,
578 and in the CCN-51 variety, Zn concentrations in leaves and beans were strongly correlated
579 (Fig. SI-2, Table SI-4). Both varieties showed strong correlations between Mn and Ba
580 concentrations in leaves, pods and beans; Ba and Zn in pods and leaves; Mn and Zn in pods
581 and between Ba and Cu in beans (Fig. SI-2, Table SI-4). The concentrations of these three
582 elements (Ba, Mn and Zn) in leaves also correlated with physicochemical soil properties:
583 negatively with pH and positively with CEC. Total organic carbon (TOC) was moderately
584 correlated with Mn, Ba and Zn in leaves in the Nacional variety (Fig. SI-2 and Table SI-4).
585 These observations suggest that soil characteristics can play a crucial role in Ba, Mn and Zn
586 absorption, by changing their chemical form and properties, and thus by extension their
587 bioavailability for cacao plants.

588 Some positive or negative correlations were also observed between TEs in topsoils and cacao
589 tissues:

590 • In the CCN-51 variety: Zn in topsoil had a positive correlation with Zn in leaves and
591 beans, and Ni in topsoil was also positively correlated with Ni in beans. Zn in topsoil
592 showed a positive correlation with TOC. High levels of soil organic matter and lower pH
593 increase the pool of bioavailable Zn in soils (Gupta et al., 2016). This result would suggest
594 that the soil clearly affected the absorption by diffusion of these two elements.

595 Passive diffusion and active transport are reported as the two main mechanisms responsible
596 for the root uptake of Zn and Ni in plants (Gupta et al., 2016). Low affinity Zn transporters
597 present at the plasma membrane (epidermal cells of roots) are also known to play a key
598 role in root Ni-uptake (Gupta et al., 2016; Shahzad et al., 2018).

599 • In the Nacional variety: Ba in topsoil showed a negative correlation with Ba in leaves and
600 beans, and Ba in topsoil correlated positively with pH (Fig SI-2 and Table SI-4),
601 highlighting a more specific active mechanism of Ba uptake. The strong ability of plants to
602 absorb Ba could be related to the Ca or K uptake (Ferreira et al., 2019; Myrvang et al.,
603 2016a, 2016b) that occurs during active or passive transport. Nevertheless, a higher pH
604 enhances Ba²⁺ sorption to Al and Fe oxyhydroxide minerals, which are difficult for plants
605 to take up (Lu et al., 2019), while a low CEC has the opposite effect (Myrvang et al.,

606 2016a). The latter is demonstrated by the strong positive correlation between CEC and Ba
607 in leaves (Fig SI-2 and Table SI-4)

608

609 **3.4.2 Potential interactions between Cd and other TEs**

610 In CCN-51, Cd concentrations in leaves were not only strongly correlated with Ni and Zn in
611 leaves but also with Cd in beans and in topsoil, respectively. In pods, Cd was correlated with
612 Cu in pods and Cd in beans. Cadmium in beans correlated with Zn and Cd in topsoil and with
613 Zn in beans (Fig. SI-2, Table SI-4).

614 In the Nacional variety, concentrations of Cd in leaves were strongly correlated with Cd in
615 beans, whereas Cd in pods was correlated with Ni and Cd in beans. The Cd content of beans
616 correlated with the Ni contents. The main differences with the CCN-51 variety were: (i) Cd
617 concentrations in topsoil correlated with Mo concentrations in pods; (ii) Cd concentrations in
618 topsoils correlated negatively with Ba contents in pods and Mn contents in beans; and (iii) the
619 correlation between Cd in topsoils and cacao tissues was low ($r < 0.5$) (Fig. SI-2, Table SI-4).

620

621 Recent studies dealing with the bioavailability of elements in soils can provide some
622 explanations, especially for Zn and Cd interactions. Cd and Zn have been reported to depress
623 and/or enhance their bioavailability to each other, but synergistic interactions have mostly
624 been observed (Gupta et al., 2019; Kabata-Pendias, 2011), perhaps because both elements
625 could share the same transporters such as ZIP proteins (Clemens et al., 2013).

626 According to Meter et al. (2019), the results of fewer correlative studies between Zn and Cd
627 levels in soil and cacao beans have so far not increased understanding of the role of Zn in
628 reducing Cd uptake. In fact, Zn fertilization has been proposed as a mitigation technique to
629 reduce Cd in cacao, as observed in other plants (Gray and Wise, 2020; Hamid et al., 2019).

630 Nevertheless, most studies of cacao have reported positive correlations between Cd and Zn in
631 cacao leaves (Blommaert, 2019; Engbersen, 2017; Gramlich et al., 2018) or between Zn in
632 beans and Cd in leaves (Arévalo-Gardini et al., 2017), suggesting that competition did not
633 play a major role in uptake. Argüello et al. (2019) found that Zn in cacao leaves was within
634 the range of adequate plant nutrition, suggesting that its deficiency was unlikely to be a factor
635 affecting Cd accumulation in cacao beans.

636 These observations are in agreement with our results, but apparently CCN-51 and Nacional
637 varieties display different mechanisms for Cd uptake. The former showed stronger
638 correlations between Zn and Cd in all cacao tissues (leaves, pod husks and beans) and in

639 topsoil, while the latter showed correlations mainly with Ni (in cacao tissues) and with Mo in
640 topsoil.

641 Recent studies of Zn and Cd isotopes in wheat had shown an opposite fractionation caused by
642 the distinct affinities of these elements to functional groups of organic ligands: Cd might be
643 bound to S ligands (glutathione or phytochelatin), whereas Zn is expected to be bound to O
644 and N functional groups (phytosiderophores or nicotianamine) (Wiggenhauser et al., 2016;
645 2018).

646 In the case of cacao plants, Cd isotopic fractionation showed either an enrichment of light
647 isotopes (Nacional variety) –similar to Zn in wheat (Wiggenhauser et al., 2018)- or a non-
648 resolvable fractionation (CCN-51 and hybrids) in beans with respect to leaves, suggesting
649 different mechanisms for root retention and/or translocation (Barraza et al., 2019).
650 Conversely, under controlled conditions, cacao clones seem to utilize similar pathways for Cd
651 sequestration but differ in their efficiency, suggesting that NRAMP5 transporters are an
652 important pathway for the uptake of Cd by cacao (Moore et al., 2020).

653 With respect to the other correlations, Cd and Ni have been reported to compete for the same
654 membrane carriers in plants (Gupta et al., 2019). An interesting observation is that the
655 positive correlation found between these two elements (in the Nacional cacao variety) and the
656 weak correlation between Cd and Zn could be explained by the fact that a reduction in Zn^{2+}
657 uptake is expected when Ni^{2+} is more abundant in the soil solution (Shahzad et al., 2018).
658 However, some authors have reported that the lack of linear correlation between Cd and Ni in
659 cacao beans was simply because each metal has an independent mechanism of absorption
660 (Romero-Estévez et al., 2019).

661 The uptake of Mo was found to increase under Cd stress, without a clear impact from Cd on
662 the Mo translocation from shoot to root (Ismael et al., 2018). This trend has been observed in
663 other plants, such as yellow monkey flower, where both elements showed a strong positive
664 relationship probably because both elements are tightly linked through Fe-homoeostasis
665 (Lowry et al., 2012).

666 Finally, this kind of correlation matrices can bring new insights for the understanding of
667 interactions between elements in the cacao mineral nutrition, depending on the cacao
668 genotypes and topsoil characteristics. It is still hard to draw conclusions, however, and further
669 studies are in progress to better identify the role of each element and how they can modulate
670 the global uptake of major and trace elements, and thus the potential contamination risk
671 through bioaccumulation processes.

672

673 **4. Conclusion**

674 The present study, conducted in a total of 31 small farms located in five different provinces of
675 Ecuador, has provided a special focus to the distribution of a cocktail of trace elements in
676 topsoils and the associated cacao plant tissues. The main results suggest that even if
677 concentrations of As, Ba, Co, Cu, Cr, Mo, Mn, Ni, Pb, V and Zn are relatively high in soils
678 (due to natural occurrence and/or anthropogenic activities), their transfer to cacao leaves, pod
679 husks and beans remains quite low.

680 Cacao varieties displayed different levels of TEs in each tissue but their effect is clearly
681 modulated in the field by physicochemical soil properties, plant physiology and perhaps also
682 by their proximity to oil facilities in the study area. The TEs concentrations in the cocoa
683 liquor samples –including Pb and Cu, both regulated by the Codex Committee- remained low
684 and safe for human consumption when compared to estimated daily intakes.

685 Although only two of the most popular cacao varieties cultivated in Ecuador were considered
686 (CCN-51 and Nacional), moderate to strong correlations and some differences have been
687 observed across some parameters, including Cd interactions with Zn and Ni. These results
688 have already been observed in other crops, since elements with the same ionic charges share
689 membrane transporters.

690 However, the use of isotopic geochemistry tools seems to be an effective method with which
691 to identify the role and the expression of those transporters, and further studies are now in
692 progress to better understand these mechanisms.

693 Finally, Cd interactions between major elements (N, P, K, Mg, S, Ca and Fe) essential for
694 cacao crops but not addressed in this study, could led to a better interpretation of their
695 interactions. Further research is thus required in order to evaluate the nutritional status of
696 cacao seedlings exposed to different levels of Cd, specifically under controlled conditions or
697 specific fertilizing amendments.

698

699

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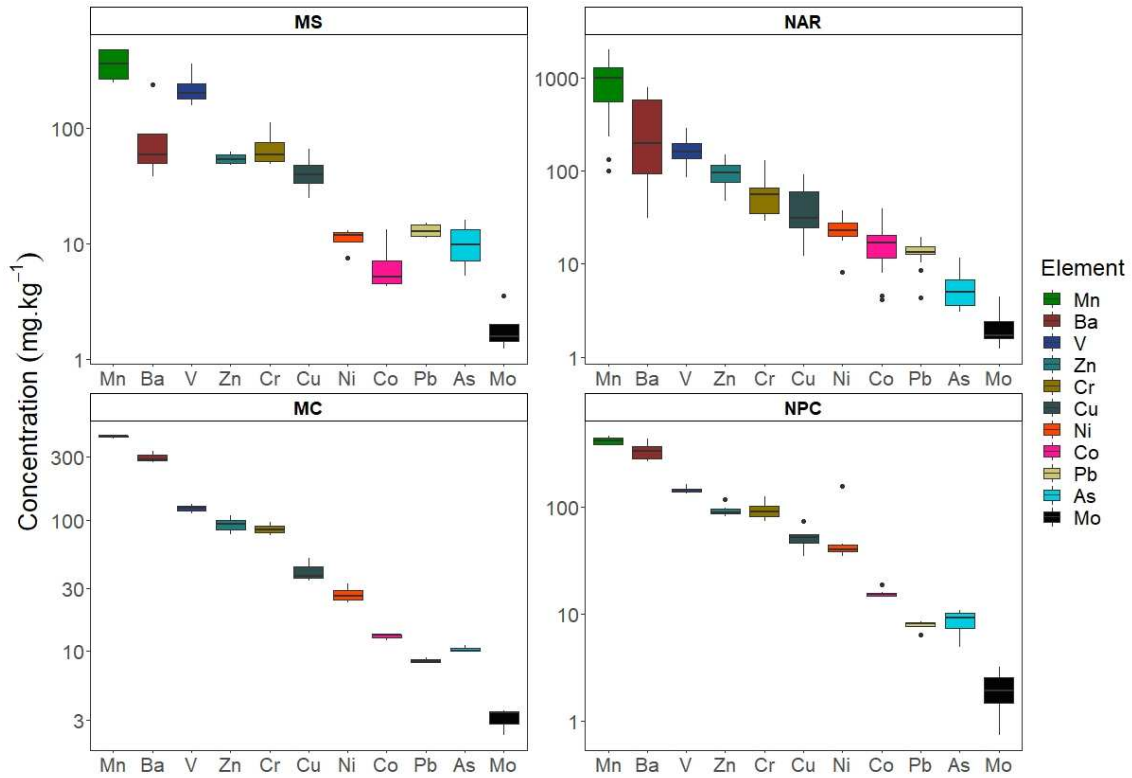


Fig. 1. Mean concentrations (mg kg⁻¹)* of Mn, Ba, V, Zn, Cr, Cu, Ni, Co, Pb, As and Mo in topsoils (0-20 cm) collected in the Ecuadorian provinces of Morona Santiago (MS: control area, free from mining and oil activities), Orellana and Sucumbíos (NAR: oil extraction), Manabí (MC: control area, free from mining activities but with intensive agriculture) and Esmeraldas (NPC: close to the main national oil refinery).

*from analytical replicates

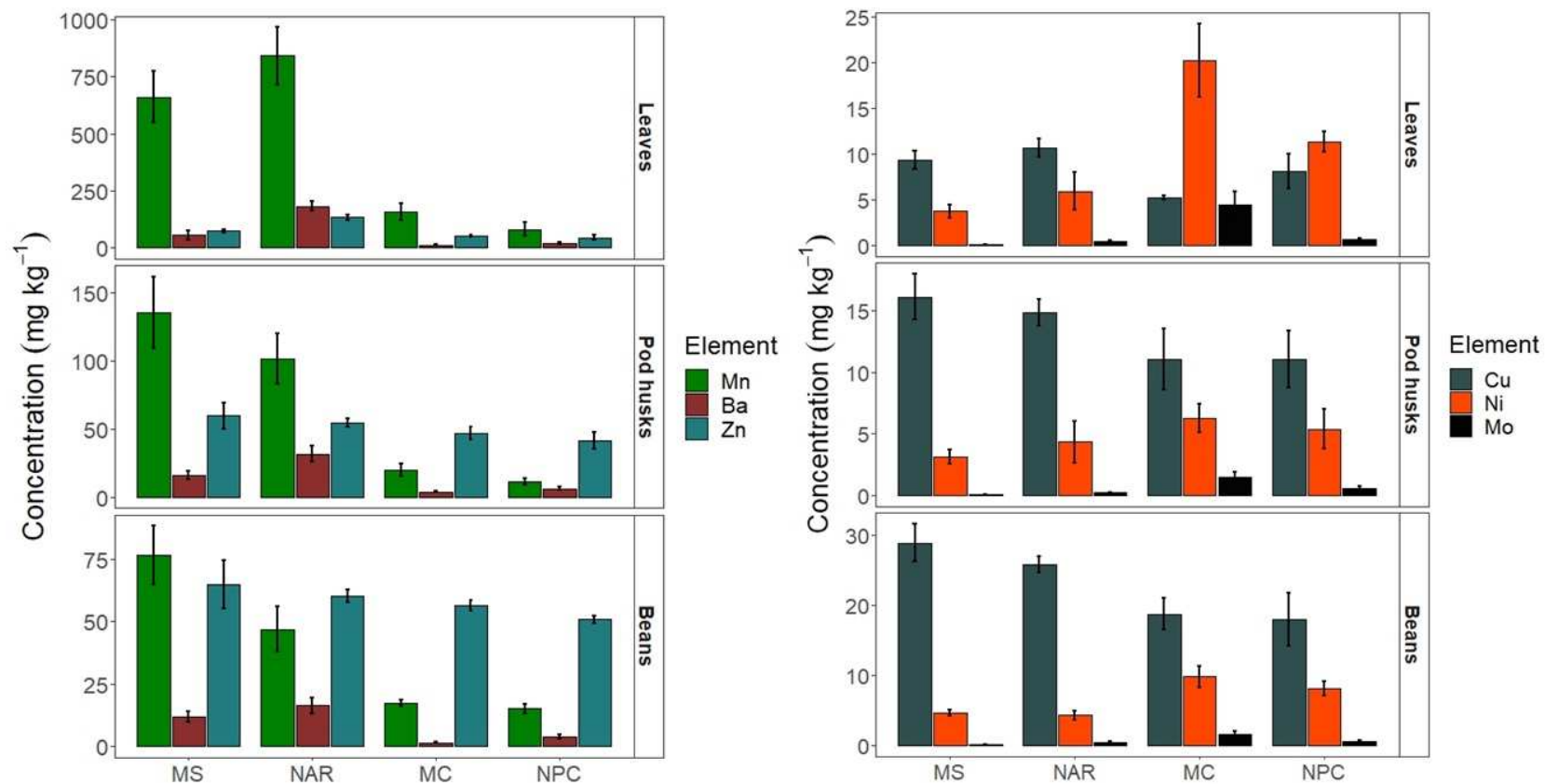


Fig. 2. Mean concentrations ($\text{mg kg}^{-1} \pm \text{SE}$) of Mn, Ba, Ni, Cu and Zn in cacao leaves, pod husks and beans from Ecuador for all varieties collected in the field and classified according to sampling area: Morona Santiago (MS: control area, free from mining and oil activities), Orellana and Sucumbíos (NAR: oil extraction), Manabí (MC: control area, free from mining activities but with intensive agriculture) and Esmeraldas (NPC: close to the main national oil refinery).

SE: standard error, calculated from SD and number of samples

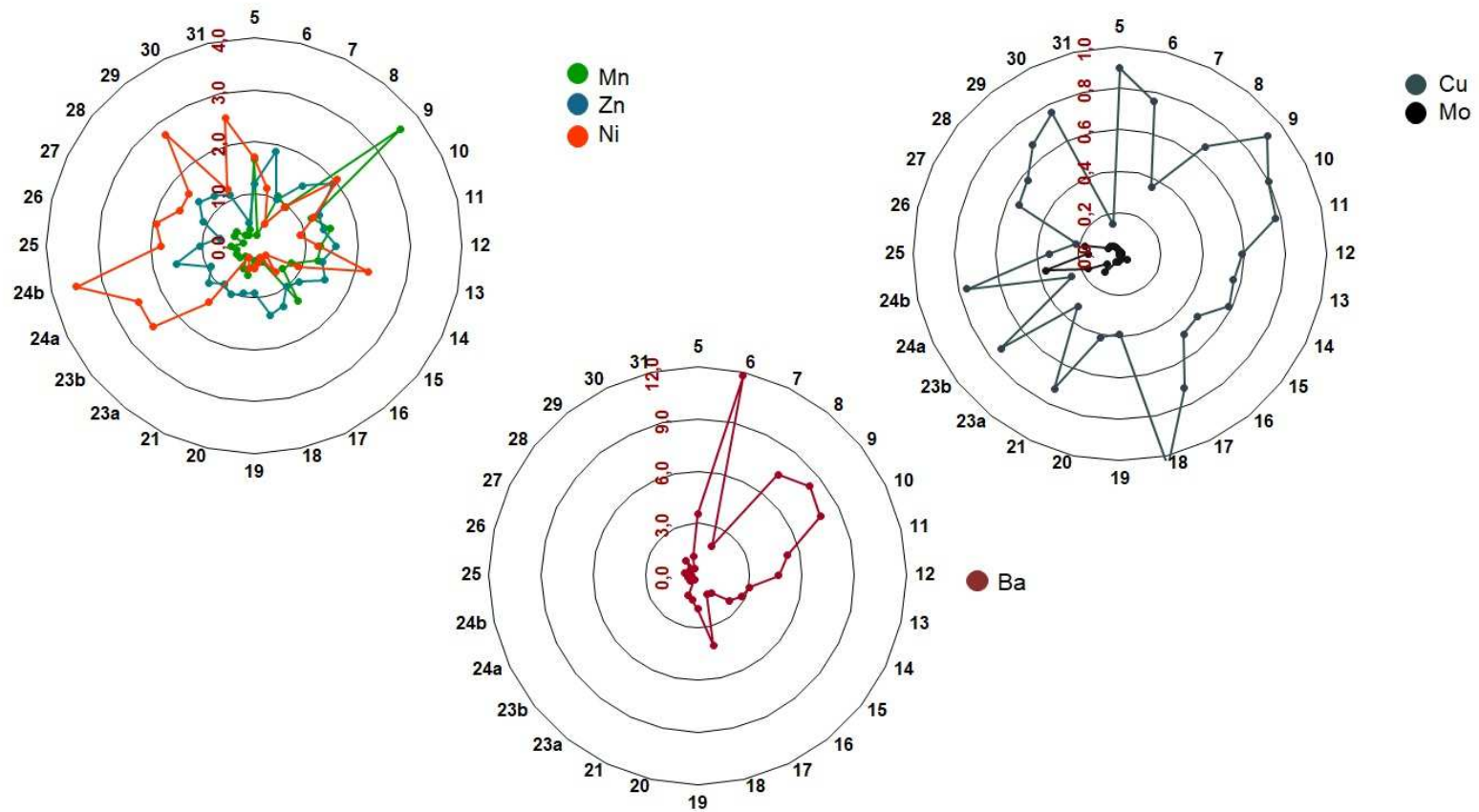


Fig. 3. Global Enrichment Factors (GEF) calculated for Mn, Zn, Cu, Mo, Ni and Ba in Ecuadorian cacao beans. Numbers outside the spider graphic (from 5 to 31) represent the sampling codes for cacao beans (for CCN-51 and Nacional varieties).

Table 1. Concentrations of trace elements (in mg kg⁻¹ ± SD, except for Pb expressed in µg kg⁻¹ ± SD) in the 2 cocoa liquor samples (CL-1 and CL-2) collected in Pacayacu, province of Sucumbíos, in the North Amazon Region (NAR) of Ecuador, compared with the CODEX Alimentarius guidelines.

Samples	Zn	Cu	Mn	Ba	Ni	Mo	Co	Pb [¥]
Codex^a	--	20 [§] ,35 [†]	--	--	--	--	--	1000 [§] ,2000 [†]
CL-1^{*.b}	54 ±3	26±0.06	18±0.2	6.68±0.06	4.38±0.02	0.93±0.02	0.55±0.03	6.08±0.30
CL-2^{*.c}	44±5.4	23±0.06	12±0.1	9.19±0.09	7.76±0.03	0.54±0.01	0.38±0.02	10.36±0.52

SD= standard deviation from lab replicates of the same sample

¥ in µg kg⁻¹

^a United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), CODEX Committee on cocoa products and chocolate (1997)

§chocolate, † unsweetened chocolate

*CCN-51 x Nacional hybrids

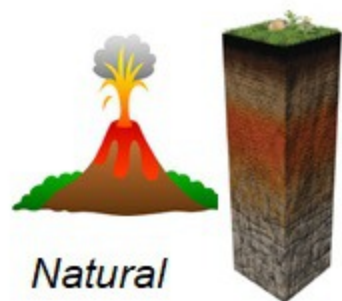
b,c different years of production

Table 2. Mean, min and max values of Transfer Factor (TF) of trace elements for cacao leaves, pod husks and beans (CCN51 and Nacional varieties, n=30).

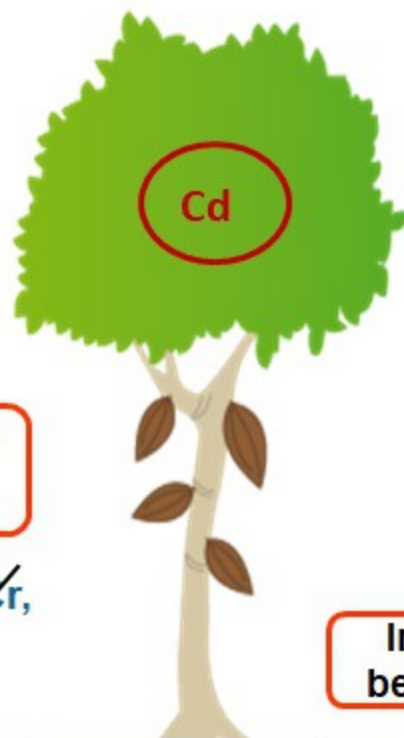
Trace elements	Essentials					Non-essential
	Mn	Zn	Cu	Mo	Ni*	Ba
Leaves						
Mean	1.42	1.21	0.26	0.51	0.35	0.96
Min	0.03	0.17	0.08	0.01	0.03	0.01
Max	14.29	2.98	0.73	3.65	1.40	5.04
Pod husks						
Mean	0.17	0.59	0.38	0.18	0.18	0.17
Min	0.01	0.23	0.09	0.02	0.02	0.01
Max	1.37	1.11	1.01	0.86	1.40	1.14
Beans						
Mean	0.09	0.68	0.66	0.24	0.21	0.09
Min	0.01	0.36	0.14	0.02	0.04	<0.01
Max	0.56	1.28	1.94	1.25	0.45	0.34

*Ni is considered as essential for several plants (Fabiano et al., 2015; Kabata-Pendias and Szteke, 2015)

Mixed- sources of trace elements (TEs)



Ecuadorian cacao



Accumulation?

Leaves > Pod husks ≥ Beans



Risks for human health?

Transfer of other
TEs?

Mn, Ba, ~~V~~, Zn, ~~Cr~~,
Cu, Ni, ~~Co~~,
~~Pb~~, ~~As~~, Mo

Interactions
between TEs?

Tropical Soil

Mineral nutrition

